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SCREENING OF RICE VARIETIES AND *IN VITRO* EVALUATION OF BOTANICALS AGAINST FALSE SMUT PATHOGEN, *USTILAGINOIDEA VIRENS*

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ABSTRACT

False smut of rice caused by the fungus Ustilaginoidea virens is becoming a major disease throughout rice growing countries of the world. For the management of this disease prophylactic application of certain fungicides are being recommended. Since false smut affects the grains directly, environment friendly approaches such as utilization of host plant resistance and use of safer plant derived products are more suitable. In the present study, 20 rice varieties were screened against false smut in the field conditions under natural disease incidence. Seven varieties viz., Ptb 7, Ptb 23, Ptb 24, Ptb 32, Ptb 36, Ptb 42 and Ptb 46 were free from disease. These can be tested in the field again for confirmation of resistance. Out of ten plant extracts (10%) tested in vitro, against Ustilaginoidea virens, bulb extract of garlic (Allium sativum), rhizome extract of turmeric (Curcuma longa), leaf extracts of lantana (Lantana camara) and bael (Aegle marmelos) inhibited the pathogen considerably. Among the five plant oils (1%) tested, lemon grass (Cymbopogon flexuous) cinnamon (Cinnamomum zeylanicum), and palmarosa (Cymbopogon martinii) oils inhibited the pathogen completely. These plant extracts and plant oils inhibitory to Ustilaginoidea virens could be utilized for the development of botanical formulations for the management of false smut.

KEYWORDS: False Smut, Ustilaginoidea Virens, Plant Extracts, Plant Oils, Rice

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INTRODUCTION

Diseases are one of the major biotic constraints of rice production causing yield loss throughout the rice growing countries of the world. Fungal, bacterial and viral diseases are affecting rice production. Among the fungal diseases blast, sheath blight and brown spot are commonly seen throughout the world. Several minor diseases of rice are becoming major threats in recent years. False smut caused by the fungus *Ustilaginoidea virens* (Cooke) Takahashi, is a sporadic disease but distributed widely (Tanaka *et al.*, 2008). Outbreak of false smut epidemics have been reported from different parts of world (Atia, 2004; Rush *et al*., 2000; Singh and Pophaly, 2010). In India, the occurrence and intensity of false smut was increased over the past few years (Ladhalakshmi, 2012). Depending on the varieties grown and disease intensity the yield loss caused by false smut was estimated to vary from 0.2 – 49 per cent (Doden and Singh, 1996). The symptoms appear only at flowering stage. The pathogen converts the grain into yellowish to green velvety spore balls resulting in both qualitative and quantitative yield loss. The false smut spores are reported to be toxic to human beings and animals because of the presence of the toxin, ustiloxin produced by the fungus (Koiso *et al*., 1994). Environmental factors favouring the disease are low

temperature particularly low night temperature, high humidity and rainfall at the time of flowering. With the increase in dose of nitrogen fertilizer increase in incidence of false smut was reported (Parsons *et al*, 2001; Mohiddin *et al.*, 2012).

For the management of disease, deployment of resistance is the most economic and safer practice. The resistance of rice genotypes to false smut under natural disease incidence was reported by various workers. (Biswas, 2001; Ahonsi *et al.*, 2000; Mohiddin *et al.*, 2012; Atia, 2004; & Singh and Singh, 2005). Since it is a relatively newer disease in many parts, the systematic screening has not been taken up and even if conducted they are mainly based on natural disease incidence in the field which is highly influenced by the environmental conditions. Since the disease affects the grains directly use of chemical fungicides as a curative measurement is not that much successful as in case of other diseases of rice, and there would be a chance of residue problems. In this context, an integrated approach of disease management would be more viable. Plant extracts is one of the best options for disease management, particularly in the context of organic farming. The efficacy of plant extracts against rice diseases have been reported by various researchers (Sehajpal *et al.*, 2009; Biswas, 2007; Dutta and Kalha, 2011; Kamalakannan *et al*., 2001 & Hubert *et al*., 2015). Use of essential oils is another ecofriendly strategy of management of diseases. The antimicrobial properties of plant oils against several fungal and bacterial pathogens has been proved (Sehajpal *et al*., 2009; Bansod and Rai, 2008; El Baroti *et al*., 2010; Alrajhi, 2014; Nguefack *et al*., 2008 & Soylu *et al*., 2006).

False smut is widely seen in predominant rice varieties that are being cultivated by the famers of Kerala State. Even though there are few fungicides for the management of the disease, as the disease appears only in the late stage of the crop, its management is very difficult. The information on host plant resistance is also lacking. In this back ground studies were conducted to assess the field resistance of few varieties to false smut and to test the effect of plant oils and plant extracts against false smut.

MATERIALS AND METHODS

Screening Rice Varieties for Resistance to False Smut

Twenty rice varieties were screened in the field to assess their resistance to false smut under natural disease pressure. Each test varieties were grown in five square meter sized plots under normal nutrient management conditions during the *kharif* season of 2013. The varieties tested were, Ptb 1, Ptb2, Ptb5, Ptb7, Ptb13, Ptb14, Ptb23, Ptb24, Ptb28, Ptb32, Ptb 34, Ptb35, Ptb36, Ptb 39, Ptb 42, Ptb46, Ptb49, Ptb50, Ptb51 and Ptb52. The per cent disease incidence was recorded as number of panicles infected per m^2 and disease severity by number of spikelets affected per panicle. Scoring was done as per the standard evaluation system (SES) scale of IRRI 2002.

Scale	Infected Florets
0	No incidence
1	Less than 1 %
3	1 – 5%
5	6 – 25%
7	26 – 50%
9	51 – 100%

In vitro Evaluation of Plant Extracts against *Ustilaginoidea virens*

Ten different plant extracts were tested to find their effectiveness against *Ustilaginoidea virens* by poisoned food

technique. 10 per cent water extracts of leaves of Catharanthus (*Catharanthus roseus*), Ocimum (*Ocimum tenuiflorum*), Adathoda (*Adathoda vesica*), Panikoorka (*Coleus aromaticus*), Bael (*Aegle marmelos*), Lantana (*Lantana camara*), Eupatorium (*Eupatorium odoratum*) and Mango *(Mangifera indica*), rhizome extract of Turmeric (*Curcuma longa)* and bulb extract of Garlic (*Allium sativum*) were tested. The plant extracts prepared were filter sterilized and added to the yeast peptone potato dextrose (YPPD) medium to get 10 per cent concentration. Control sets were prepared by adding sterilized distilled water instead of plant extracts. Seven days old fungal culture discs of 9 mm size were placed at the centre of the plated medium in the petri plates and incubated at room temperature. Four replications were maintained for each treatment. The diameter of fungal growth was recorded eight days after incubation. The analysis of variance was performed and means were separated by Fischer's LSD Test.

In vitro* Evaluation of Essential Oils against *Ustilaginoidea virens

An *in vitro* study was carried out to test the efficacy of five plant oils *viz.*, Lemongrass oil (*Cymbopogon flexuous)*, Cinnamon oil (*Cinnamomum zeylanicum*), Palmarosa oil (*Cymbopogon martinii*), Vetiver oil (*Chrysopogon zizanioides*) and Maroti oil (*Hydnocarpus pentadra)* against *Ustilaginoidea virens* by poisoned food technique. To prepare one per cent concentration of the oils, 1ml oil was mixed with 5 ml of 1 % filter sterilized Tween 80 and then added to 95 ml of YPPD medium and plated. The control sets were prepared by adding sterilized distilled water instead oils. Seven days old fungal culture discs of 9 mm size were placed at the centre of the petriplate and incubated at room temperature. Four replications were maintained for each treatment. Observation on radial growth was recorded on the eighth day. The per cent reduction in radial growth over the control was calculated. Measurement on radial growth of the fungus were square root transformed. The analysis of variance was performed and means were separated by Fischer's LSD test.

RESULTS AND DISCUSSIONS

The incidence of false smut ranged from 0 to 31.43 per cent. The spikelet infection percentage ranged from 0 to 7.20 per cent (Table 1). The 20 varieties fall into four groups of standard evaluation system based on infected florets per panicle (Table 2). Among the 20 rice varieties screened for false smut resistance, in the field, seven varieties *viz.*, Ptb7, Ptb23, Ptb24, Ptb32, Ptb36, Ptb42 and Ptb 46 showed no incidence of disease. Four varieties were grouped under 2 with score 1. The floret infection percentage ranged from 0.80 per cent to 0.97 per cent. The disease incidence percentage of these four varieties ranged from 0.5 to7.17 per cent. Eight varieties falls to the third category with score of 3. These were Ptb 1, Ptb 2, Ptb 28, Ptb 34, Ptb 35, Ptb 39, Ptb 49, Ptb 51. The spikelet infection per cent ranged from 1.23 per cent to 5.10 per cent and disease incidence ranged from 0.43 to 15.95 per cent. The group 4 comprised one variety Ptb 52 with score 5. The percentage of infected florets were 7.20 and the incidence was 31.43.

Attempts were made to screen rice varieties for resistance to false smut by different researchers. Mohiddin *et al* (2012) screened four rice genotypes for resistance to false smut. Among the four genotypes screened, HRI 119 recorded lowest disease incidence. Kaur *et al*. (2015) evaluated 125 rice genotypes comprising hybrids and inbred lines by inoculating the plants by spraying the spore suspension on emerging panicles in which they got nine hybrids with no disease incidence. Singh and Singh (2005) screened 98 rice genotypes against false smut of which 27 were found resistant. Lore *et al* (2013) grouped 25 rice hybrids in to five groups based on false smut score. Yan *et al*.(2014) screened 186 rice hybrids and located few hybrids with low disease incidence.

Eventhough the host plant resistance is the most economical way of management of false smut, at present predominantly cultivated high yielding varieties of Kerala State are susceptible to false smut. In the present study among the rice varieties tested seven varieties were free from the disease and these could be further evalauted to confirm the resistance.

In the present study, among the 10 plant extracts tested, garlic recorded the highest inhibition (57.6%) of false smut pathogen. This was followed by Turmeric (44%) Lantana (41.6%) and Bael (30.8%) (Table3). The Adathoda leaf extract enhanced the growth of false smut pathogen. Plant products are viable alternatives or supplements to chemicals for plant disease management. Several plant products were found inhibitory to rice pathogens also. The inhibitory effect of garlic against *Rhizoctonia solani*, causing sheath blight of rice was reported by Sehajpal *et al.* (2009). The antimicrobial property of turmeric is well documented. The effect of turmeric on seed born rice diseases was reported by Gangopadhyay (1998). The seed treatment with turmeric powder plus sodium carbonate in the ratio 100:10 @ 1g per kg rice seeds, and foliar spray of turmeric impregnated sodium carbonate (1g l^{-1}) at maximum tillering stage resulted in a reduction in severity of symptoms of *Helminthosporium oryzae, Rhizoctonia solani, Acrocylindrium oryzae, Pyricularia grisea, Curvularia lunata* and *Xanthomonas oryzae* pv *oryzae*. Datta and Kalha (2011) found the inhibitory effect of bael (*Aegle marmelos*) and *Lantana camara* against *Rhizoctonia solani*, the sheath blight pathogen of rice.

Among the five plant oils tested, lemon grass oil (*Cymbopogon flexuous),* cinnamon oil (*Cinnamomum zeylanicum*) and palmarosa oil (*Cymbopogon martinii*) inhibited the growth of *Ustilaginoidea virens* completely. The other three oils had no inhibition of *U. virens*. The vetiver oil (*Chrysopogon zizanioides*) enhanced the growth of *Ustilaginoidea virens in vitro*. The antifungal properties of plant oils and their use in disease management of various crops have been reported (Wilson *et al*., 1995, Soylu *et al.,* 2006 & Nguefack, 2008). The antimicrobial property of cinnamon oil (*Cymbopogon citratus*) against rice pathogens *Alternaria padwickii*, *Bipolaris oryzae* and *Fusarium moniliforme* was reported by Nguefack *et al.,* (2008). The inhibition of fungal pathogens of cinnamon by cinnamon oil and Lemon grass oil was reported (Alrajhi, 2014). The inhibition of mycelial growth of *Sarocladium oryzae in vitro* and reduction in sheath rot severity in field by citronella oil was reported (Sharma *et al*., 2013). The antifungal activity of essential oil of *Cymbopogon martinii* against anthracnose pathogens of banana, *Colletotrichum musae* and *Botryodiplodia theobrome* was reported by Muthukumar and Ranganathan (2012).

CONCLUSIONS

In the field screening, seven rice varieties (Ptb 7, Ptb 23, Ptb 24, Ptb 32, Ptb 36, Ptb 42 and Ptb 46) were free from false smut. The resistance of these seven varities could be confirmed by further screening. The inhibitory plant extracts *viz*., garlic, turmeric, bael, lantana, and plant oils *viz*., lemon grass oil, cinnamon oil and palmarosa oil reported from this study could be utilised for the development of botanical fungicides against false smut

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APPENDICES

Table 1: Reaction of Different Rice Varieties to False Smut Disease

Sl No	Varieties	Incidence of False Smut (%)	Spikelet Infected per Panicle (%)
1	Ptb1 (Aryan)	15.95	5.10
2	Ptb2 (Ponnaryan)	8.50	1.50
3	Ptb5 (Velutharikayama)	7.17	0.80
4	Ptb 7 (Chuvannarithavalakkannan)	0.00	0.00
5	Ptb13 (Kayama)	4.80	0.95
6	Ptb14 (Maskathi)	0.50	0.97
7	Ptb 23 (Velutharyan)	0.00	0.00
8	Ptb 24 (Chuvannavattan)	0.00	0.00
9	Ptb28 (Kattamodan)	0.43	1.47
10	Ptb 32 (Aruvakkari)	0.00	0.00
11	Ptb34 (Valiyachampan)	1.17	1.23
12	Ptb35 (Annapoorna)	1.97	4.26

Table 1: Contd.,			
13	Ptb 36 ((Rohini)	0.00	0.00
14	Ptb39 (Jyothi)	5.23	2.30
15	Ptb 42(Suvarnnamodan)	0.00	0.00
16	Ptb 46 (Jayathi)	0.00	0.00
17	Ptb49 (Kairali)	1.67	3.50
18	Ptb50 (Kanchana)	1.00	0.98
19	Ptb51 (Athira)	6.04	4.71
20	Ptb52 (Aiswarya)	31.43	7.20

Table 2: Grouping of Genotypes Based on False Smut Disease

Group	Genotypes	Disease Score
1	Ptb 7, Ptb 23, Ptb 24, Ptb 32, Ptb 36, Ptb 42, Ptb 46	0
2	Ptb 5, Ptb 13, Ptb 14, Ptb 50	1
3	Ptb 1, Ptb 2, Ptb 28, Ptb 34, Ptb 35, Ptb 39, Ptb 49, Ptb 51	3
5	Ptb 52	5

Table 3: Effect of Plant Extracts on *Ustilaginoidea virens*

Treatments	Diameter of Fungal Growth (cm)	Percent Inhibition
T1 - Catharanthus (*Catharanthus roseus*)	1.85^{de}	26.0
T2 - Ocimum (*Ocimum tenuiflorum*)	2.16^{bcd}	13.6
T3 - Adathoda (*Adathoda vesica*)	3.75^{a}	-50.0
T4 - Panikoorka (*Coleus aromaticus*)	2.30^{cd}	0.08
T5 - Turmeric (*Curcuma longa*)	1.40^{fg}	44.0
T6 - Garlic (*Allium sativum*)	1.06^{g}	57.6
T7 - Bael (*Aegle marmelos*)	1.73^{def}	30.8
T8 - Lantana (*Lantana camara*)	1.46^{efg}	41.6
T9 - Eupatorium (*Eupatorium odoratum)*	2.44^{bc}	2.4
T10 - Mango *(Mangifera indica*)	2.40^{bc}	4.0
T11 - Control	2.50^{b}	0.0
CD (0.05%)	0.43	

Each value is a mean of four replications. In the same column means followed by the same letter are not significantly different (P= 05) according to Fischer's LSD test.

Table 4: Effect of Plant Oils on *Ustilaginoidea Virens*

Treatments	Diameter of Fungal Growth (cm)	Percent Inhibition
T_{1}- Lemongrass oil (*Cymbopogon flexuous*)	$0\ (1.00)^{c}$	100
T_{2}- Cinnamon oil (*Cinnamomum zeylanicum*)	$0\ (1.00)^{c}$	100
T_{3}- Palmarosa oil (*Cymbopogon martinii*)	$0\ (1.00)^{c}$	100
T_{4}- Vetiver oil (*Chrysopogon zizanioides*)	$3.38\ (2.09)^{a}$	-53.64
T_{5}- Maroti oil (*Hydnocarpus pentadra)*	$2.14\ (1.77)^{b}$	2.72
T_{6}- Untreated control	$2.20\ (1.79)^{b}$	0
CD (0.05%)	0.046	

Values in parenthesis are $\sqrt{(x+1)}$ transformed. Each value is a mean of four replications. In the same column means followed by the same letter are not significantly different (P= 05) according to Fischer's LSD test.